BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Jan Zavada et al.

Technology Center: 1600

Serial No.: 08/260,190

Group Art Unit: 1633

Filed : June 15, 1994

Examiner: Dave Nguyen

For : MN Gene and Protein

DECLARATION CONCERNING MN CDNA SEQUENCE AND
AMINO ACID SEQUENCE DEDUCED THEREFROM
WITH ATTACHED APPENDICES 1 AND 2

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

We, Drs. Jan Zavada, Jaromir Pastorek and Silvia Pastorekova, declare as follows.

1. That we are the inventors of the invention disclosed and claimed in the above-identified application.

2. Dr. Zavada is on the research faculty at the Institute of Molecular Genetics of the Academy of Sciences of the Czech Republic in Prague. Drs. Pastorek and Pastorekova are on the research faculty at the Institute of Virology of the Slovak Academy of Sciences in Bratislava, Slovakia. However, at the time the inventions described in the above-identified application were made, we were all members of the research faculty at the Institute of Virology.

3. There are errors in the MN cDNA sequences and amino acid sequences deduced therefrom as set forth in the instant application and in the applications, from which the instant application claims priority, those priority application being U.S. Serial No. 07/964,589 [filed on October 21, 1992; issued as U.S. Patent No. 5,387,676 on February 7, 1995], and commonly owned, copending U.S. Serial No. 08/177,093 (filed on December

30, 1993). An incorrect partial MN cDNA [1397 base pairs (bp)] and deduced partial amino acid sequence [429 amino acids (a.a.)], missing the putative signal peptide, are shown in the instant application and priority applications in Figure 1A-1B and are designated SEQ ID NO: 1 and SEQ ID NO: 2, respectively. Further, an incorrect full-length MN cDNA sequence [1519 bp] and deduced full-length MN amino acid sequence [466 a.a.] are shown in the instant application and in its parent application [U.S. Serial No. 08/177,093] in Figure 15 and are designated SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

4. Most of the errors in the MN cDNA sequence and deduced amino acid sequence identified in paragraph 3, above, were due to nonapparent compressions of nucleotides, especially of Gs and Cs, which were initially unrecognized during sequencing of the MN cDNA. Such compressions are often known to be the source of errors in nucleic acid sequencing. Those initially nonapparent nucleotide compressions were recognized after filing the instant application, when the results of resequencing the partial and full-length MN cDNAs (derived from HeLa cells) were compared with the results of the earlier sequencing of those same full-length and partial MN cDNA clones and with the results from sequencing MN genomic clones (derived from fetal brain cells). The same sequencing protocols were used for resequencing as were originally used to sequence the partial MN cDNA clone (the MN14 clone) to which Zavada et al., U.S. Patent No. 5,387,676 refers at column 11, lines 54-56, and to which the instant application refers at page 38, lines 5-11.

5. That partial MN cDNA clone (identified as the MN14 clone) was resequenced, and errors were found in the original sequencing results as detailed infra. That partial MN cDNA clone (the MN14 clone), when sequenced correctly, has the correct partial MN cDNA sequence of SEQ ID NO: 1 (as corrected) of the instant application. The isolation and sequencing of that partial MN cDNA clone (the MN14 clone) are described at page 37, line 15 to page 38, line 11 of the instant application and grandparent application [U.S. Serial No. 07/964,589, filed October 21, 1992 (now U.S. Patent No. 5,387,676 at column 11, lines 41-65)]. Briefly, a lambda gt11 cDNA library was prepared from the total mRNA isolated from MX-infected HeLa cells [i.e., HeLa cells infected with lymphocytic choriomeningitis virus

(LCMV)]. That cDNA library was immunoscreened, according to the method described in Young and Davis, PNAS (USA), 80: 1194-1198 (1983), using the M75 monoclonal antibody produced by the VU-M75 hybridoma, which was deposited at the American Type Culture Collection (ATCC) on September 17, 1992. One positive clone was picked (as described in U.S. Patent No. 5,387,676 at column 11, lines 54-56 and in the instant application at page 38, lines 5-11) and subcloned into the NotI site of pBluescript KS [Stratagene; La Jolla, CA] to create pBluescript-MN. Serial bidirectional nested deletions were made using an Erase-a-BaseTM kit [Promega; Madison, WI (USA)] and sequenced by the dideoxynucleotide chain termination method using a T7 sequencing kit [Pharmacia; Piscataway, NJ (USA)]. The sequencing showed a partial cDNA clone, the insert being 1397 bp long.

6. The grandparent application [U.S. Serial No. 07/964,589 (filed October 21, 1992)] to which the instant application claims priority, is now U.S. Patent No. 5,387,676. That Zavada et al. patent indicates at column 12, lines 4-8, and the instant application at page 39, lines 3-6 notes: "[A]s follows from a comparison of the size of the MN clone with that of the corresponding mRNA in a Northern blot (Figure 4), the cDNA was missing about 100 bp from the 5' end of its sequence." Attempts to isolate a full-length cDNA clone from the lambda gt11 cDNA library failed. As described in the parent application to the instant application [U.S. Serial No. 08/177,093 (filed December 30, 1993)] at page 33, line 13 to page 34, line 14, and in the instant application at page 39, line 7 to page 40, line 36, we performed a rapid amplification of cDNA ends (RACE) using MN-specific primers derived from the 5' region of the original partial cDNA clone (MN14), to which U.S. Patent No. 5,377,676 and the instant application refer. RACE was performed using 5' RACE System [GIBCO BRL; Gaithersburg, MD (USA)]. The RACE product was inserted into pBluescript II KS (Stratagene), and the entire population of recombinant plasmids was sequenced with the MN-specific primer ODN1, which is SEQ ID NO: 3 (a 29-mer) disclosed in the grandparent [U.S. Serial No. 07/964,589 (filed October 21, 1992); now U.S. Patent No. 5,387,676] and in the instant application. In that way, we obtained the sequence at the very 5' end of the MN cDNA. A full-length MN cDNA clone, identified as the flMN clone, was obtained by ligation of the MN14 cDNA and

the RACE clone product, both cleaved in the unique BamHI site. That full-length MN cDNA clone (the flMN clone), when sequenced correctly, has the nt sequence of SEQ ID NO: 5 of the instant application.

7. As explained in the instant application at page 41, lines 1-12, MN genomic clones were then isolated from a human cosmid library prepared from fetal brain using a MN cDNA probe and MN-specific primers derived from the 5' end of the cDNA, which primers are disclosed in the instant application and in the grandparent application [U.S. Serial No. 07/964,589 (filed October 21, 1992); now U.S. Patent No. 5,387,676] as SEQ ID NOS: 3 and 4, ODN1 (29-mer) and ODN2 (19-mer).

8. As explained in the commonly owned, co-pending continuation-in-part applications, e.g., U.S. Serial No. 08/477,504 (filed June 7, 1995) at page 42, line 7 to page 44, line 4, to identify the complete genomic region of MN, a human genomic library in Lambda FIX II vector (Stratagene), was prepared from HeLa chromosomal DNA and screened by plaque hybridization using MN cDNA. Several independent MN recombinant phages were identified, isolated and characterized by restriction mapping and hybridization analyses. Four overlapping recombinants covering the whole genomic region of MN were selected, digested and subcloned in pBluescript KS. The genomic clones were sequenced and relevant parts of the MN cDNA sequence were resequenced. Templates for sequencing were generated by serial bidirectional nested deletions using an Erase-a-Base™ Kit (Promega). Sequencing was performed by the dideoxynucleotide chain termination method using a T7 sequencing kit (Pharmacia). Thus, we used the same sequencing protocols to sequence the partial MN cDNA (to which U.S. Patent No. 5,387,676 and the instant application refer; the MN14 clone) as we did to sequence the genomic clones and to resequence the partial MN cDNA clone the MN14 clone) and the full-length MN cDNA clone (the flMN clone). Nucleotide sequence alignments and analyses were carried out using the DNASIS software package (Hitachi Software Engineering).

9. By sequencing and resequencing the genomic clones of the MN gene, by repeated sequencing of relevant parts of the partial MN cDNA clone (to which the instant application and U.S. Patent No. 5,387,676 refer; the MN14 clone) and the full-length

MN cDNA clone (the flMN clone), and by comparing the sequencing results obtained, we recognized that there were mistakes made in sequencing the partial cDNA clone (the MN14 clone) and full-length MN cDNA clone (the flMN clone). As indicated above and explained further below in paragraph 12, most of those mistakes occurred due to nonapparent compressions of nucleotides, especially of Gs and Cs, that often are a source of sequencing errors in that they are difficult to identify.

10. We believe, based upon the analysis of the above-described sequencing results, that the MN cDNA sequence, which comprises 1522 bp and is shown as the top sequence in Appendix 1 attached hereto is a correct and complete full-length MN cDNA sequence. SEQ ID NO: 5 (as corrected) of the instant application is the nt sequence of the full length MN cDNA clone (flMN). That true and complete MN cDNA sequence of 1522 bp differs from the partial MN cDNA sequence of the instant application [SEQ ID NO: 1], of the parent application [U.S. Serial No. 08/177,093 (filed December 30, 1993)], and of the grandparent application [U.S. Serial No. 07/964,589 (filed October 21, 1992; now U.S. Patent 5,387,676], in that the partial MN cDNA of 1397 bp is missing the 5' 123 nucleotides (nts) and contains a number of errors. The sequence alignment of Appendix 1 clearly identifies those errors. SEQ ID NO: 1 (as corrected) of the instant application is the nt sequence of the partial MN cDNA clone (MN14).

11. The correct full-length MN cDNA sequence of 1522 bp is shown in parallel with the incorrect full-length MN cDNA sequence in the accompanying Appendix 1, wherein the top sequence of the alignment is the correct cDNA sequence (the "NEW MNCDNA.SEQ") and the bottom sequence is the missequenced MN cDNA sequence (the "OLD MN.SEQ"). The instant application and its parent application (U.S. Serial No.: 08/177,093) designate the full-length MN cDNA sequence as SEQ ID NO: 5 and show it in Figure 15. The partial MN cDNA sequence of the instant application and its grandparent and parent applications [SEQ ID NO: 1] begins at nucleotide (nt) position 124 of the bottom sequence of the alignment. The sequence alignment shows the sequencing errors at the following positions, wherein the positions are stated according to the corrected full-length MN cDNA sequence:

- at position 159, there is an insertion of one G;
- at position 308, there is a deletion of one T;
- at position 454, there is an insertion of one C;
- at positions 599-601, GCA replaces AG;
- at position 674, there is insertion of one G;
- at position 839, there is an insertion of one C;
- at position 875, there is a deletion of one T; and
- at position 1357, there is a deletion of one G.

12. Most of the errors delineated above were due to GC compressions. An exception is the error concerning the substitution of GCA for AG at positions 599-601 of the corrected sequence; that error was simply the result of an error in the sequence reading. However, the deletions of T at positions 308 and 875 did occur as a result of GC compressions for the following reason. Because of the GC compressions, we originally recognized two Gs instead of three Gs at two different sites. As a consequence of that erroneous reading, the open reading frame (ORF) moved to a position -1 (with respect to the correct ORF), and a stop codon appeared several nucleotides thereafter. That reading would have resulted in smaller proteins having molecular weights of about 12.6 kilodaltons (kd) and 22.5 kd, respectively; however, there were tiny T-like bands at positions 308 and 875. Such weak bands readily occur on sequencing films, and if they are very close to each other (on the bottom of a film), it is sometimes difficult to recognize whether the distance to a neighboring band is correct. In our original sequencing work, those false Ts fit very well in the original sequence because they shifted the position of the ORF to the correct phase, and the resulting protein product was of the required length.

13. We clearly and unequivocally confirm that we resequenced correctly the partial MN cDNA clone (the MN14 clone) to which U.S. Patent No. 5,387,676 refers at column 11, lines 54-56 and to which the instant application refers at page 38, lines 5-11, and the full-length MN cDNA clone (the flMN clone), which were originally sequenced incorrectly, and identified the nt sequence of those clones with the correct partial and full-length MN cDNA sequences -- SEQ ID NOS: 1 and 5, respectively of the instant application as corrected. We were always dealing with the same partial MN cDNA clone (MN14) and full length MN cDNA clone (flMN). The MN gene is a single copy gene. From our

viewpoint, it would not have made sense to admit publicly our sequencing errors if we had in our possession two MN cDNA clones having different sequences. Further, we were always using the M75 monoclonal antibody (mab) which recognizes an epitopic region of the MN protein affected by the sequence corrections. The M75 mab would certainly not bind specifically to the protein expressed from an MN cDNA clone with the incorrect sequence. The fact that the protein expressed from the MN cDNA clone, to which U.S. Patent No. 5,387,676 and the instant application refer (the MN 14 clone), and from the full length MN cDNA clone (the flMN clone) was identified by the M75 mab, and still reacts with the M75 mab, confirms that that cDNA had been originally missequenced, and that we had always been working with the same cDNA.

14. As a consequence of the errors in originally sequencing the partial and full-length MN cDNA sequences (of the MN14 and flMN clones), areas of the amino acid sequences deduced from the incorrect cDNA sequences are incorrect. The incorrect deduced partial amino acid sequence is disclosed in the instant application and in the parent and grandparent applications as SEQ ID NO: 2 (429 a.a.), and is shown in Figure 1A-1B. The incorrect deduced full-length MN amino acid sequence is disclosed in the instant application and in its parent application (U.S. Serial No.: 08/177,093) as SEQ ID NO: 6 and shown in Figure 15. The differences between the incorrect partial and full-length amino acid sequences [SEQ ID NO: 2 of the instant application, parent and grandparent applications, and SEQ ID NO: 6 of the instant and parent applications, respectively], and the corrected full length amino acid sequence, which contains 459 amino acids and is deduced from the correct full-length MN cDNA sequence of the flMN clone, is shown in the sequence alignment attached as Appendix 2. The top sequence in Appendix 2 labeled "MN.AMI" is the incorrect full-length MN amino acid sequence deduced from the incorrect full-length MN cDNA sequence. The partial MN amino acid sequence, begins at amino acid 38 of the full-length MN amino acid sequence. The bottom sequence of the Appendix 2 alignment labeled "NEW MN.AMI" is the true and complete full-length deduced MN amino acid sequence.

15. We hereby declare further that all statements made herein of our own knowledge are true, and that all statements

made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Dated: 08/27/1999

JAN ZAVADA

Dated: 08/20/1999

JAROMIR PASTOREK

Dated: 08/20/1999

SILVIA PASTOREKOVA

Attorney's Docket No.: D-0021-2

COMBINED DECLARATION AND POWER OF ATTORNEY

As the below named inventor(s), I (we) hereby declare that:

My (Our) residence, post office address and citizenship(s) are as stated below next to my (our) name(s).

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled MN GENE AND PROTEIN the specification of which is attached herewith.

I (We) hereby state that I (we) have reviewed and understand the contents of the above-identified specification, including claims.

I(We) acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, section 1.56.

I (We) hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Serial No.	Filing Date	Status
08/177,093	December 30, 1993	Pending
07/964,589	October 21, 1992	Pending

I (We) hereby claim foreign priority benefits under Title 35, United States Code Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

			Priority claimed Yes	No
PV-709-92	Czechoslovakia	03/11/92	/X/	/ /
Number	Country	Filing Date		

*****

POWER OF ATTORNEY:

As the named inventor(s), I (we) hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Names and Registration Nos.:

Leona L. Lauder, Registration No. 30,863

Please send all correspondence to:

Leona L. Lauder
Attorney at Law
177 Post Street, Suite 800
San Francisco, CA 94108

Please direct all telephone calls to:

Leona L. Lauder
(415) 421-4973

I(We) hereby declare that all statements made herein of my own (our) knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Jan Zavada
Full Name of Inventor

Jan Zavada
Signature

Prague, Czech Republic
Residence (City, State or Foreign Country)

June 26, 1994
Date

Na pekne vyhlidce 1
16000 Prague 6, Czech Republic
Postal Address (Street, City, State, Zip)

Czech Republic
Citizenship

Silvia Pastorekova
Full Name of Inventor

Silvia Pastorek
Signature

Bratislava, Slovak Republic
Residence (City, State or Foreign Country)

6/26/94
Date

I. Bukovcana 18, 84107
Bratislava, Slovak Republic
Postal Address (Street, City, State, Zip)

Slovak Republic
Citizenship

Jaromir Pastorek
Full Name of Inventor

Jaromir Pastorek
Signature

Bratislava, Slovak Republic
Residence (City, State or Foreign Country)

6/26/94
Date

I. Bukovcana 18, 84107
Bratislava, Slovak Republic
Postal Address (Street, City, State, Zip)

Slovak Republic
Citizenship